Fig.1

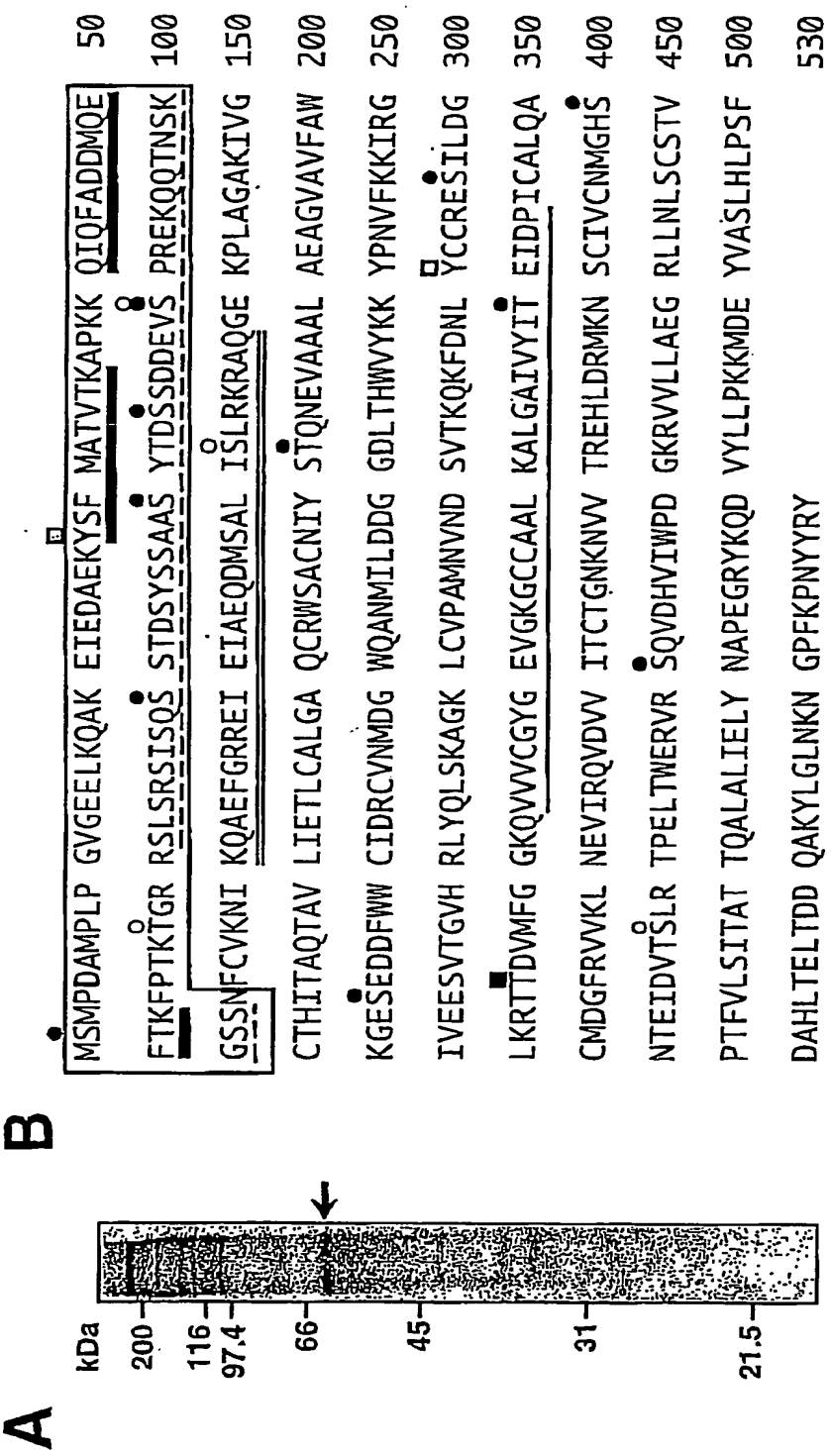

A
kDa
200
116
97.4
66
45
31
21.5
B
MSMPDAMPLP GVGEELKQAK EIEDAEKYSF MATVTKAPKK QIQFADDMQE 50
FTKFPTKTGR RSLSRSISQS STDSYSSAAS YTDSSDDEVS PREKQQTNSK 100
GSSNFCVKNI KQAEFGRREI EIAEQDMSAL ISLRKRAQGE KPLAGAKIVG 150
CTHITAQTAV LIETLCALGA QCRWSACNIY STQNEVAAAL AEAGVAVFAW 200
KGESEDDFWW CIDRCVNMDG WQANMILDDG GDLTHWVYKK YPNVFKKIRG 250
IVEESVTGVH RLYQLSKAGK LCVPAMNVND SVTKQKFDNL YCCRESILDG 300
LKRTTDVMFG GKQVVVCGYG EVGKGCCAAL KALGAIVYIT EIDPICALQA 350
CMDGFRVVKL NEVIRQVDVV ITCTGNKNVV TREHLDRMKN SCIVCNMGHS 400
NTEIDVTSLR TPELTWERVR SQVDHVIWPD GKRVVLLAEG RLLNLSCSTV 450
PTFVLSITAT TQALALIELY NAPEGRYKQD VYLLPKKMDE YVASLHLPSF 500
DAHLTELTDD QAKYLGLNKN GPFKPNYYRY 530

Fig.1

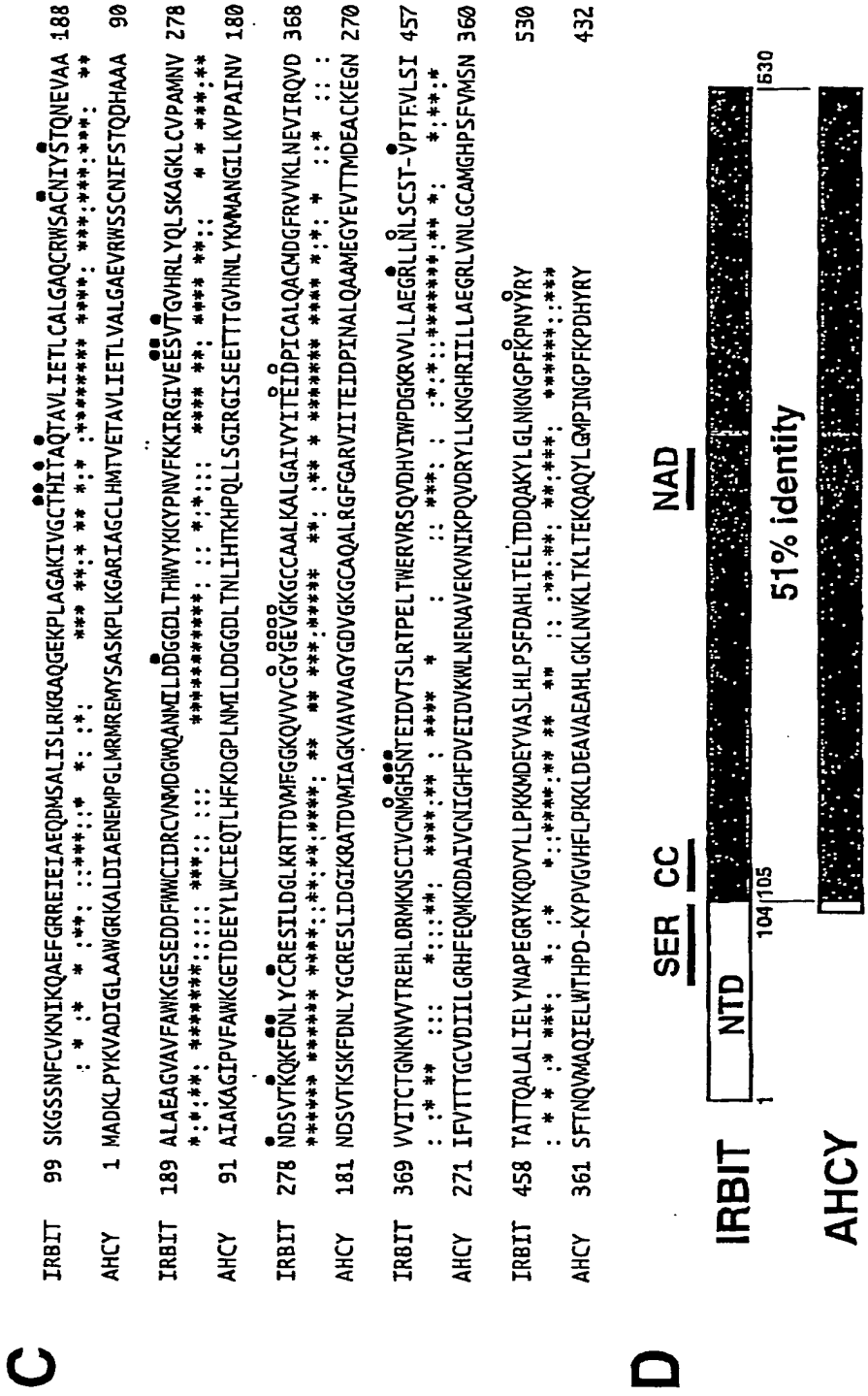

C
IRBIT 99 SKGSSNFCVKNIKQAEFGRREIEIAEQDMSALISLRKRAQGEKPLAGAKIVGCTHITAQTAVLIETLCALGAQCRWSACNIYSTQNEVAA 188
AHCY 1 MADKLPYKVADIGLAAWGRKALDIAENEMPGLMRMREMYSASKPLKGARIAGCLHMTVETAVLIETLVALGAEVRWSSCNIFSTQDHAAA 90
IRBIT 189 ALAEAGVAVFAWKGESEDDFWWCIDRCVNMDGWQANMILDDGGDLTHWVYKKYPNVFKKIRGIVEESVTGVHRLYQLSKAGKLCVPAMNV 278
AHCY 91 AIAKAGIPVFAWKGETDEEYLWCIEQTLHFKDGPLNMILDDGGDLTNLIHTKHPQLLSGIRGISEETTTGVHNLYKMMANGILKVPAINV 180
IRBIT 278 NDSVTKQKFDNLYCCRESILDGLKRTTDVMFGGKQVVVCGYGEVGKGCCAALKALGAIVYITEIDPICALQACMDGFRVVKLNEVIRQVD 368
AHCY 181 NDSVTKSKFDNLYGCRESLIDGIKRATDVMIAGKVAVVAGYGDVGKGCAQALRGFGARVIITEIDPINALQAAMEGYEVTTMDEACKEGN 270
IRBIT 369 VVITCTGNKNVVTREHLDRMKNSCIVCNMGHSNTEIDVTSLRTPELTWERVRSQVDHVIWPDGKRVVLLAEGRLLNLSCST-VPTFVLSI 457
AHCY 271 IFVTTTGCVDIILGRHFEQMKDDAIVCNIGHFDVEIDVKWLNENAVEKVNIKPQVDRYLLKNGHRIILLAEGRLVNLGCAMGHPSFVMSN 360
IRBIT 458 TATTQALALIELYNAPEGRYKQDVYLLPKKMDEYVASLHLPSFDAHLTELTDDQAKYLGLNKNGPFKPNYYRY 530
AHCY 361 SFTNQVMAQIELWTHPD-KYPVGVHFLPKKLDEAVAEAHLGKLNVKLTKLTEKQAQYLGMPINGPFKPDHYRY 432
D
SER CC
NAD
IRBIT
NTD
1
104
105
630
51% identity
AHCY

Fig.2

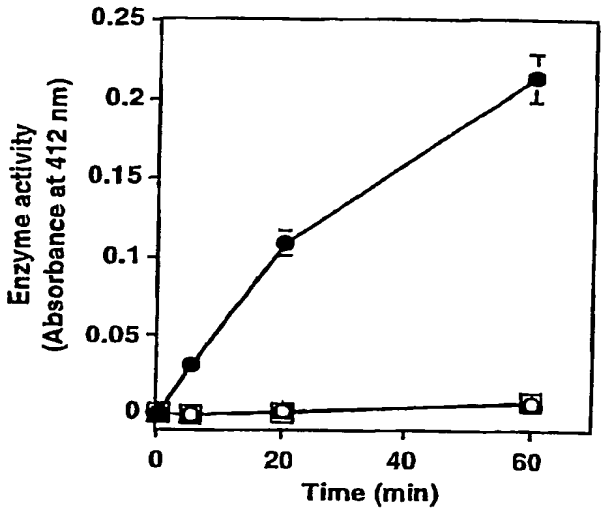

0.25
0.2
0.15
0.1
0.05
0
Enzyme activity
(Absorbance at 412 nm)
0
20
40
60
Time (min)

Fig.3

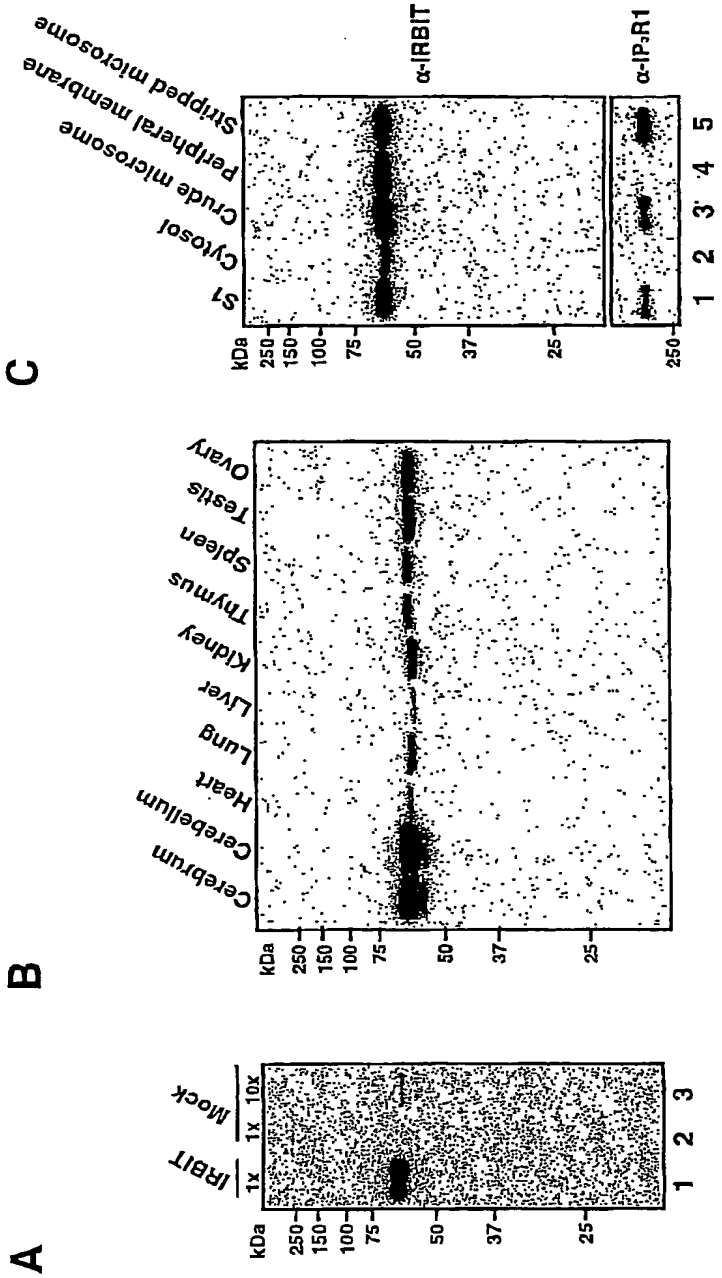

A
IRBIT
1x
Mock
1x
10x
1 2 3
kDa
250
150
100
75
50
37
25
B
Cerebrum
Cerebellum
Heart
Lung
Liver
Kidney
Thymus
Spleen
Testis
Ovary
kDa
250
150
100
75
50
37
25
C
S1
Cytosol
Crude microsome
Peripheral membrane
Stripped microsome
α-IRBIT
α-IP3R1
1 2 3 4 5
kDa
250
150
100
75
50
37
25
250

Fig.4

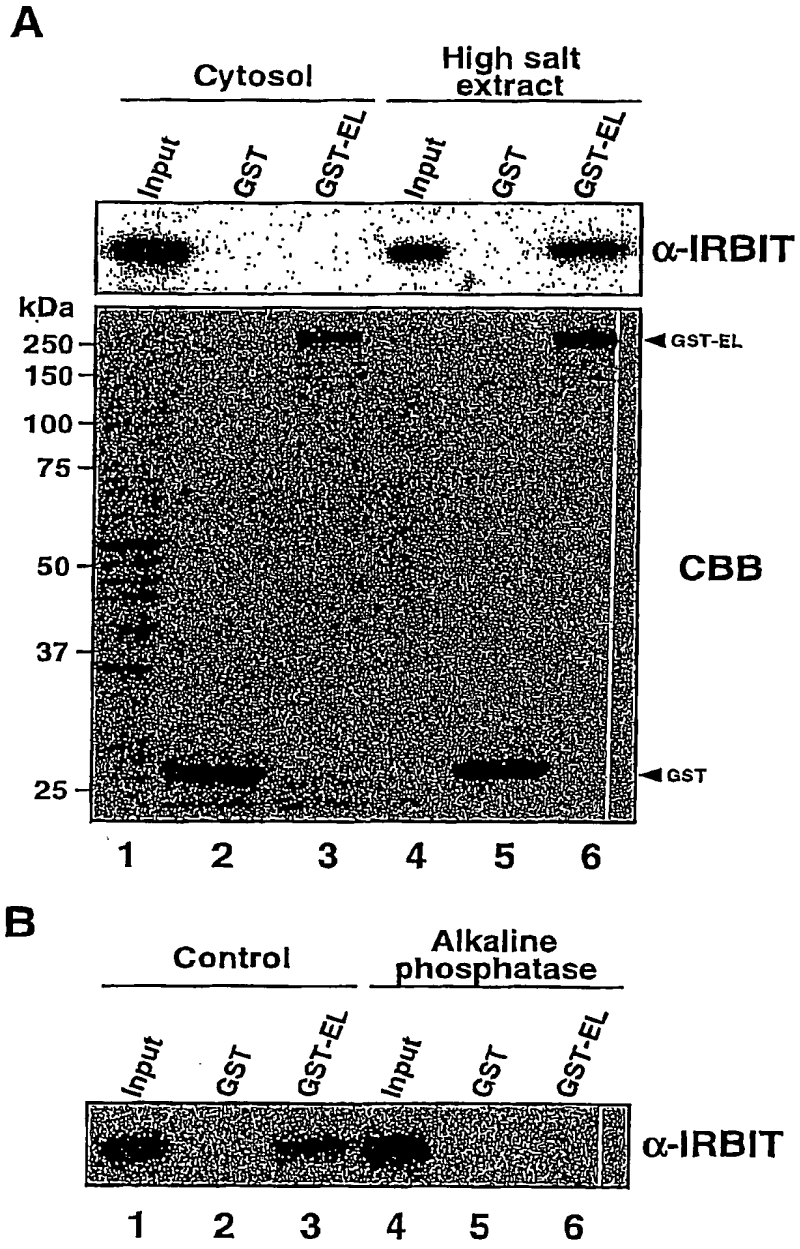

A
Cytosol
High salt extract
Input
GST
GST-EL
Input
GST
GST-EL
α-IRBIT
kDa
250
150
100
75
50
37
25
GST-EL
CBB
GST
1
2
3
4
5
6
B
Control
Alkaline phosphatase
Input
GST
GST-EL
Input
GST
GST-EL
α-IRBIT
1
2
3
4
5
6

Fig.5

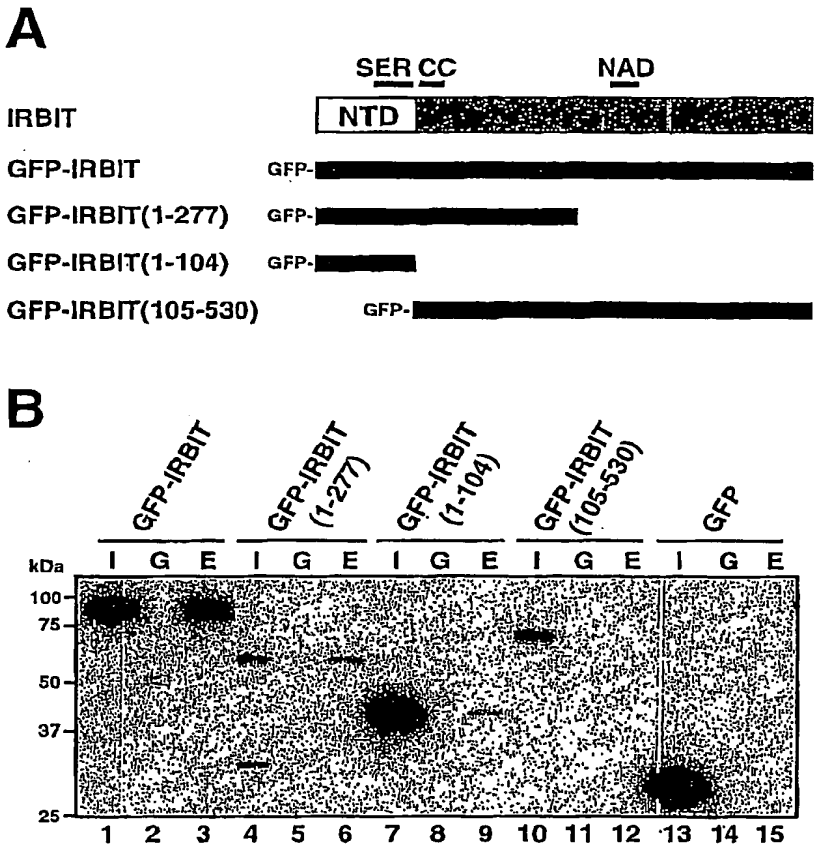

A
SER CC
NAD
IRBIT
NTD
GFP-IRBIT
GFP-
GFP-IRBIT(1-277)
GFP-
GFP-IRBIT(1-104)
GFP-
GFP-IRBIT(105-530)
GFP-
B
GFP-IRBIT
GFP-IRBIT (1-277)
GFP-IRBIT (1-104)
GFP-IRBIT (105-530)
GFP
I G E I G E I G E I G E I G E
kDa
100
75
50
37
25
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Fig.6

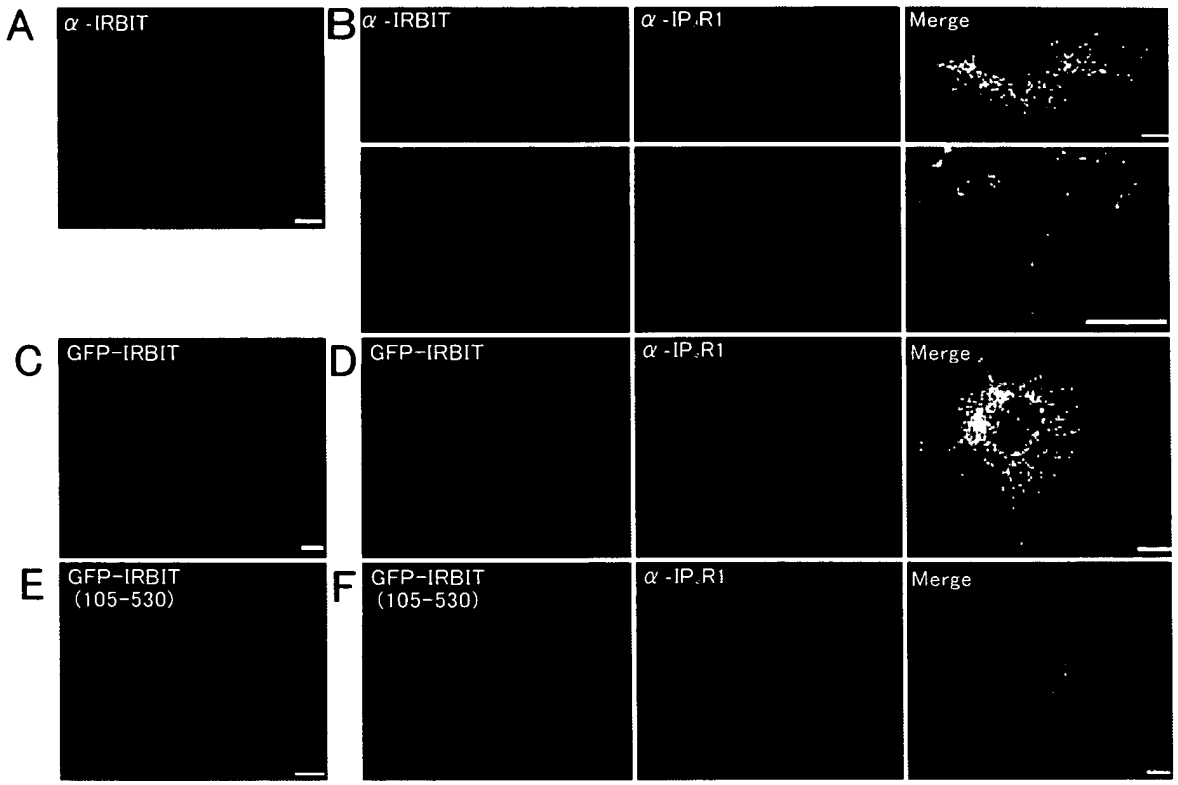

A
α-IRBIT
B
α-IRBIT
α-IP3R1
Merge
C
GFP-IRBIT
D
GFP-IRBIT
α-IP3R1
Merge
E
GFP-IRBIT
(105-530)
F
GFP-IRBIT
(105-530)
α-IP3R1
Merge

Fig.7

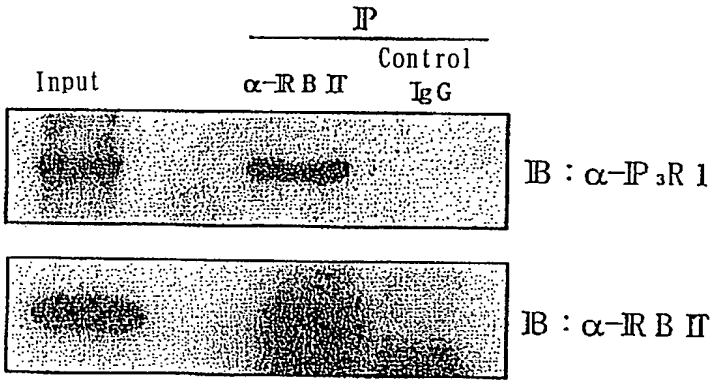


Fig.8

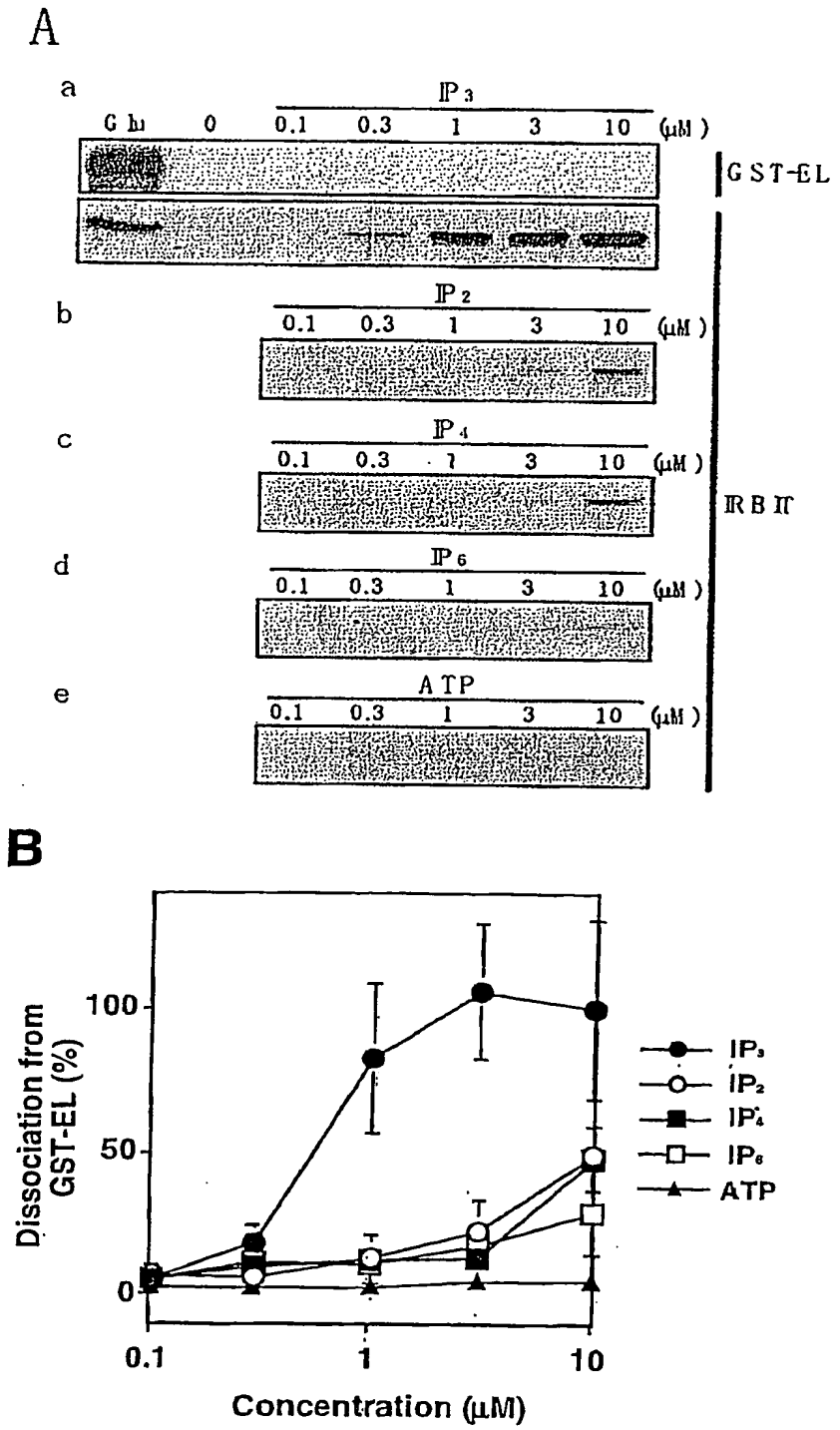

A
a
IP3
Glu 0 0.1 0.3 1 3 10 (μM)
GST-EL
b
IP2
0.1 0.3 1 3 10 (μM)
c
IP4
0.1 0.3 1 3 10 (μM)
IRBIT
d
IP6
0.1 0.3 1 3 10 (μM)
e
ATP
0.1 0.3 1 3 10 (μM)
B
Dissociation from GST-EL (%)
100
50
0
0.1
1
10
Concentration (μM)
IP3
IP2
IP4
IP6
ATP

Fig.9

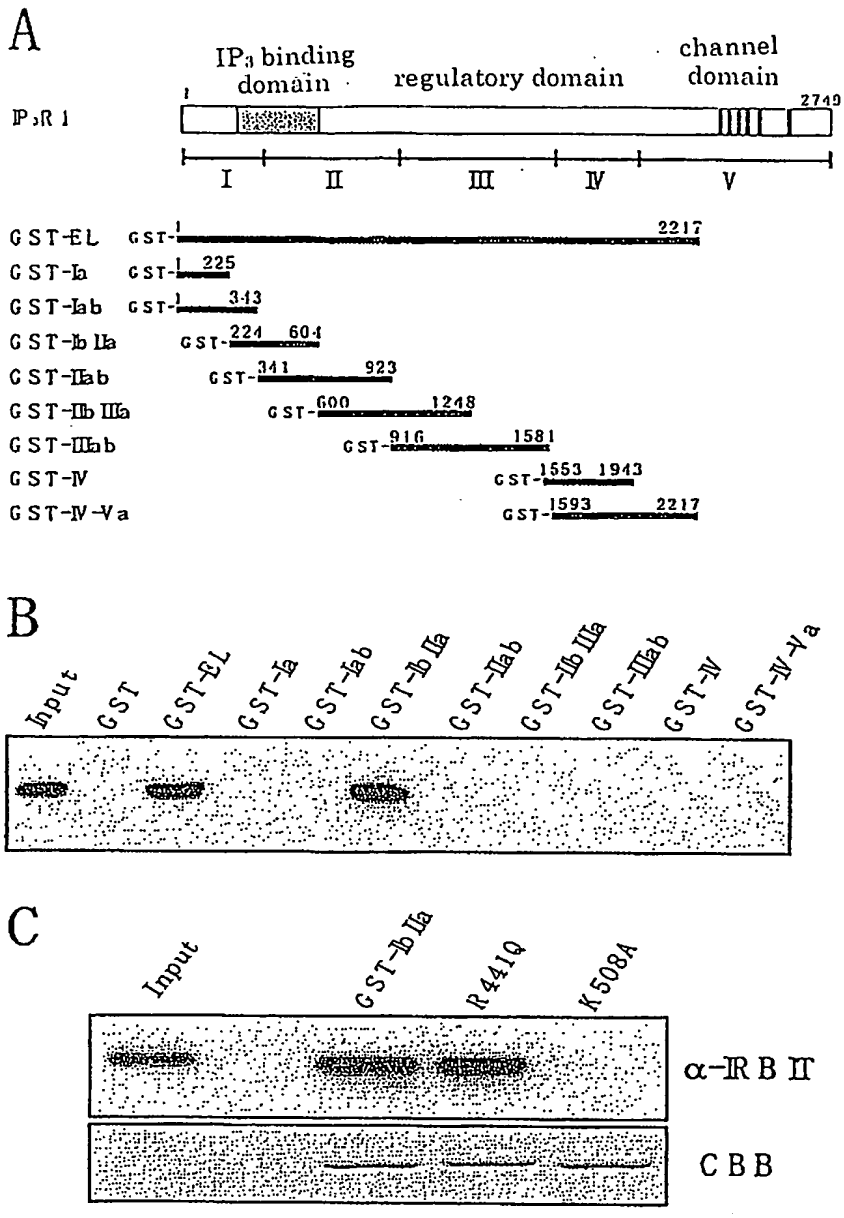
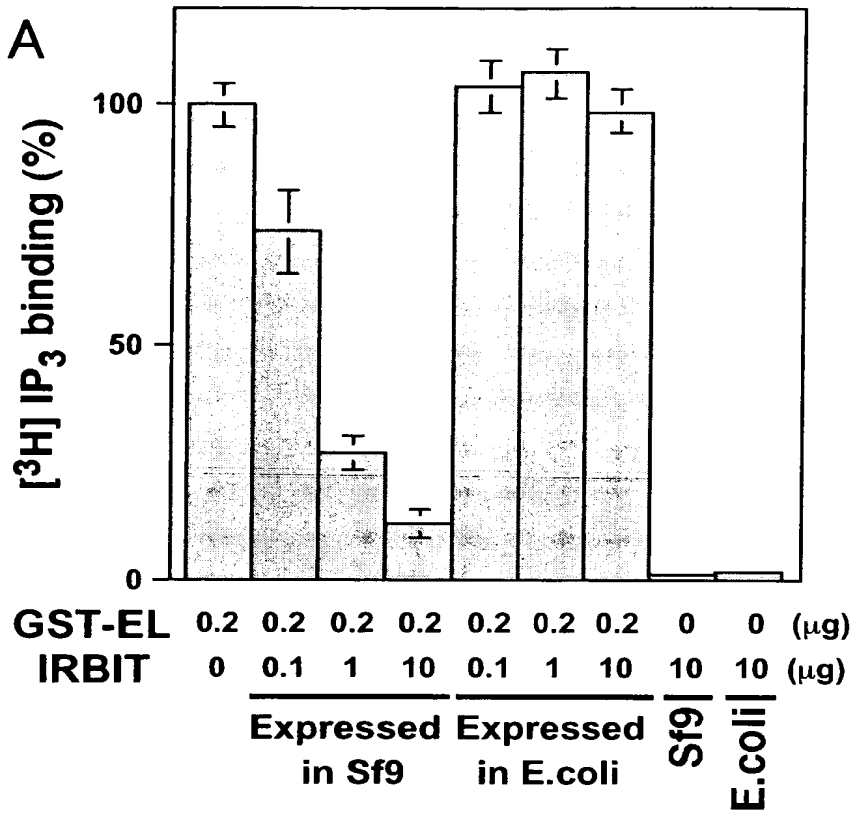

A
IP3 binding domain
regulatory domain
channel domain
IP3R 1
1
2749
I
II
III
IV
V
GST-EL GST- 1 2217
GST-Ia GST- 1 225
GST-Iab GST- 1 343
GST-Ib IIa GST- 224 604
GST-IIab GST- 341 923
GST-Ib IIIa GST- 600 1248
GST-IIIab GST- 916 1581
GST-IV GST- 1553 1943
GST-IV-Va GST- 1593 2217
B
Input
GST
GST-EL
GST-Ia
GST-Iab
GST-Ib IIa
GST-IIab
GST-Ib IIIa
GST-IIIab
GST-IV
GST-IV-Va
C
Input
GST-Ib IIa
R441Q
K508A
α-IRB II
CBB

Fig.10


A
[3H] IP3 binding (%)
100
50
0
GST-EL 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0 0 (μg)
IRBIT 0 0.1 1 10 0.1 1 10 10 10 (μg)
Expressed in Sf9
Expressed in E.coli
Sf9
E.coli

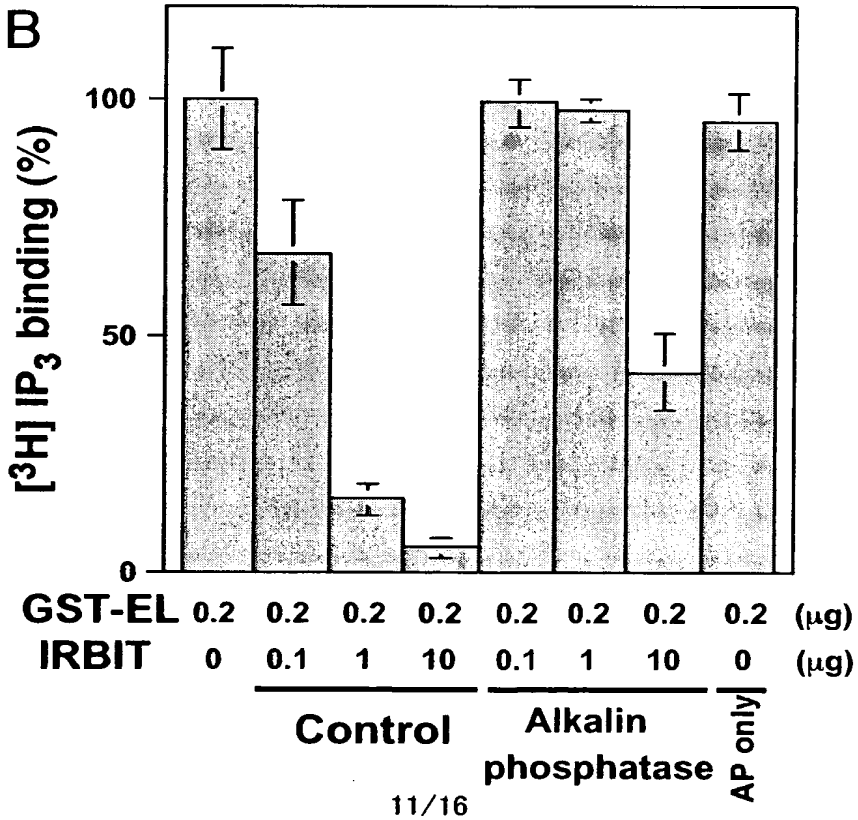
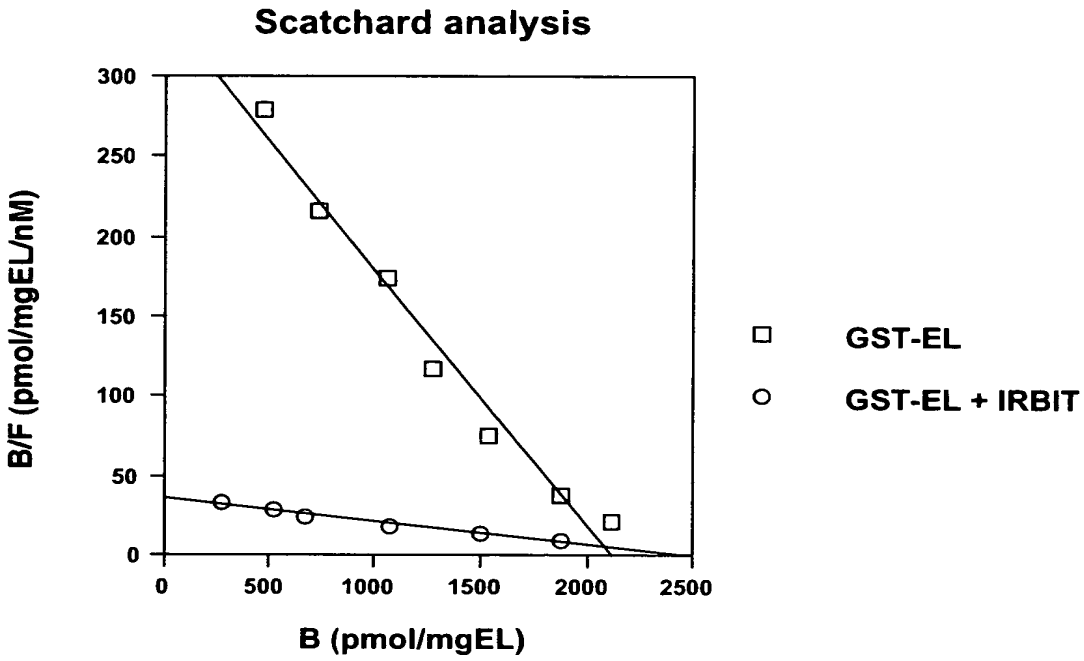

B
[3H] IP3 binding (%)
100
50
0
GST-EL 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 (μg)
IRBIT 0 0.1 1 10 0.1 1 10 0 (μg)
Control
Alkalin phosphatase
AP only

Fig.10

C



D

	GST-EL	GST-EL + IRBIT
Kd (nM)	6.08 ± 0.13	87.77 ± 20.39
Bmax (pmol/mg)	2336.5 ± 237.2	2785.3 ± 361.7

Fig.11A

no siRNA
911
911mut
490
p44mut
IRBIT
IP3R1
IP3R2
IP3R3
SERCA2
Calnexin
β-actin

Fig.11B

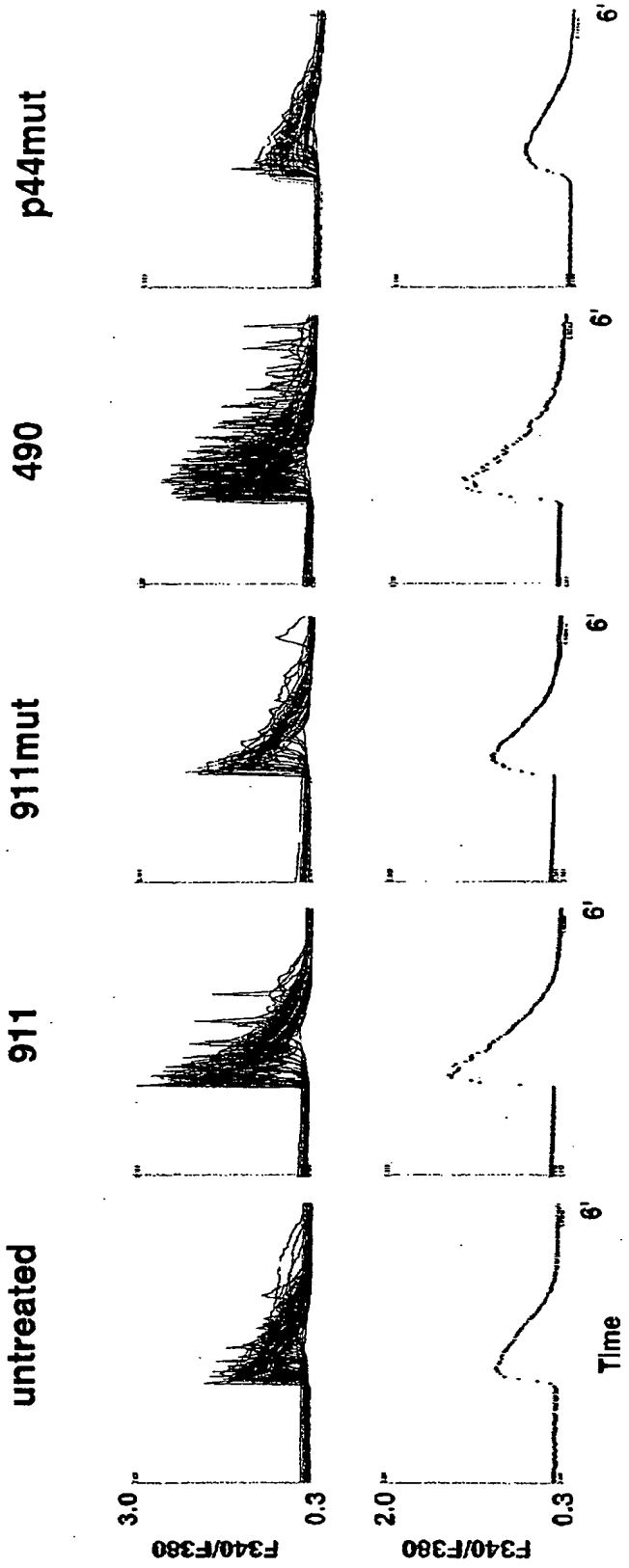

p44mut
490
911mut
911
untreated
6'
3.0
0.3
2.0
0.3
F340/F380
F340/F380
Time

Fig.12

Immunoprecipitation from COS-7 cells overexpressing
IRBIT and Myc-PIPKIIα, β, or γ

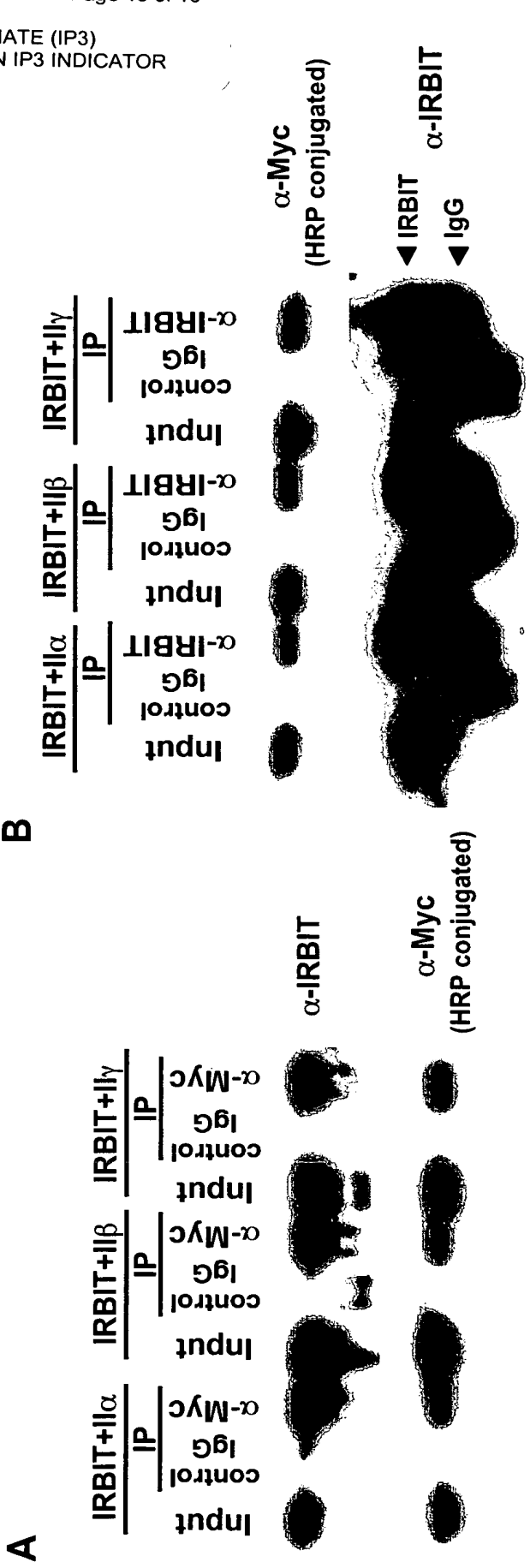
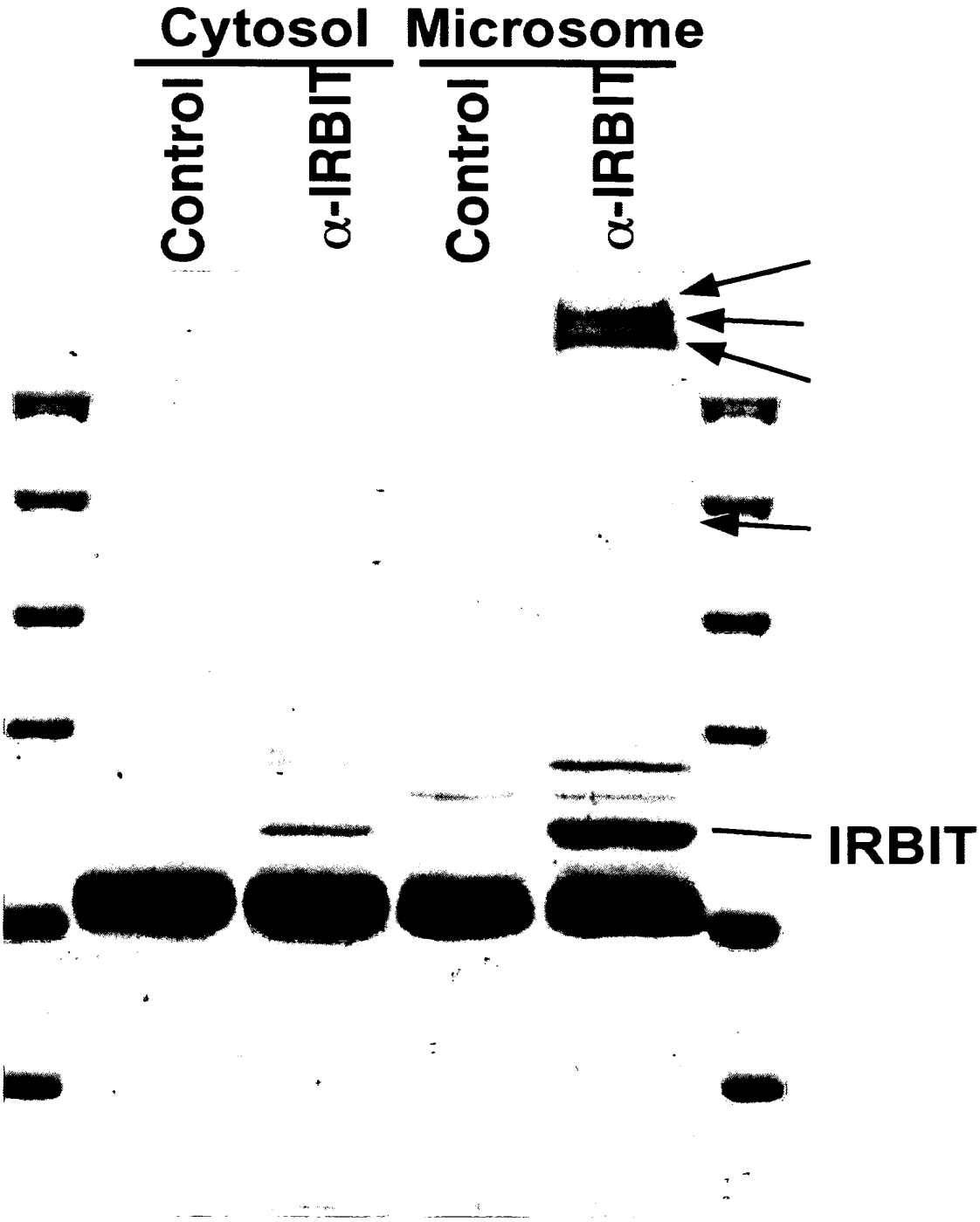



Fig.13